L-Lactate (L-LA) Content Assay Kit (WST-1 Method)

Cat. No. KITG-075 **Lot. No.** (See product label)

SPECIFICATION

Product Overview	Lactate is an important intermediate product in biological metabolism and has multiple physiological and biological functions. It is both the end product of the glycolytic energy-supply system and an oxidative substrate for the aerobic metabolic energy-supply system, and it plays important roles in energy metabolism, redox signaling, and lipid metabolism. Lactate content can serve as an important indicator for evaluating aerobic metabolism and glycogen metabolism and has broad application and research value in medicine, agriculture, industry, and food science. L-lactate dehydrogenase (L-LDH) catalyzes the conversion of L-lactate to pyruvate while reducing NAD^+ to NADH and H^+. Through the hydrogen-transfer action of 1-mPMS, WST-1 is subsequently reduced to water-soluble formazan. The product has a characteristic absorption peak at 450 nm, and the L-lactate content can therefore be quantitatively determined from the change in absorbance.
Description	Method: Visible spectrophotometry. Pack Size: 120 tests / 50 samples. For Research Use Only. Not for Use in Diagnostic Procedures.
Form	Kit
Applications	Quantitative determination of L-lactate content in tissue, bacterial or cell samples, serum/plasma, culture media, and other liquid samples. Lactate content can be used as an indicator for evaluating aerobic metabolism and glycogen metabolism and has research applications in medicine, agriculture, industry, and food science.
Notes	Instructions for Use Required Instruments and Reagents: Visible spectrophotometer;

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1 mL glass cuvette with a 10 mm optical path, 3 mm slit, and 1.05 mL volume; mortar/homogenizer; adjustable pipettes; benchtop centrifuge; thermostatic water bath/incubator; and distilled water. 1. Preparation of Samples to Be Tested The sample amount and ratio may be adjusted appropriately according to preliminary experimental results. 1) Tissue Process the sample at a ratio of tissue mass (g) to Extraction Solution A volume (mL) of 1:(5-10). It is recommended to weigh 0.1 g tissue and add 1 mL Extraction Solution A. Homogenize in an ice bath. Centrifuge at 12,000 × g and 4°C for 10 min. Transfer 800 µL of the supernatant to a centrifuge tube, add 150 µL Extraction Solution B, and mix thoroughly until no bubbles remain. Centrifuge at 12,000 × g and 4°C for 10 min. Keep the supernatant on ice for the assay. 2) Bacteria or Cells Collect bacteria or cells in a centrifuge tube by centrifugation. Process the sample at a ratio of bacterial or cell number (10^4) to Extraction Solution A volume (mL) of (500-1000):1. It is recommended to add 1 mL Extraction Solution A to 5 million bacteria or cells. Disrupt the bacteria or cells by sonication in an ice bath at 300 W, with 3 s sonication and 7 s intervals, for a total time of 3 min. Centrifuge at 12,000 × g and 4°C for 10 min. Transfer 800 µL of the supernatant to a centrifuge tube, add 150 µL Extraction Solution B, and mix thoroughly until no bubbles remain. Centrifuge at 12,000 × g and 4°C for 10 min. Keep the supernatant on ice for the assay. 3) Serum, Plasma, Culture Medium, and Other Liquid Samples Add 100 µL of the liquid sample to 1 mL Extraction Solution A. Centrifuge at 12,000 × g and 4°C for 10 min. Transfer 800 µL of the supernatant to a centrifuge tube, add 150 µL Extraction Solution B, and mix thoroughly until no bubbles remain. Centrifuge at 12,000 × g and 4°C for 10 min. Keep the supernatant on ice for the assay. Note: Addition of Extraction Solution B produces a large amount of bubbles. Add it slowly and mix by pipetting until no bubbles remain. A 2 mL centrifuge tube is recommended. 2. Assay Procedure 1) Preheat the spectrophotometer for at least 30 min, set the wavelength to 450 nm, and zero the instrument with distilled water. 2) Preparation of Standard Dilutions (prepare immediately before use) Dilute the 100 µmol/mL L-lactate standard solution with

distilled water to prepare standard dilutions of 0.8, 0.4, 0.2, 0.1, 0.05, and 0.025 μmol/mL as follows: A: Mix 100 μL of the 100 μmol/mL standard solution with 900 μL distilled water to obtain 10 μmol/mL. 1: Mix 80 μL of the 10 μmol/mL solution with 920 μL distilled water to obtain 0.8 μmol/mL. 2: Mix 500 μL of the 0.8 μmol/mL solution with 500 μL distilled water to obtain 0.4 μmol/mL. 3: Mix 500 μL of the 0.4 μmol/mL solution with 500 μL distilled water to obtain 0.2 μmol/mL. 4: Mix 500 μL of the 0.2 μmol/mL solution with 500 μL distilled water to obtain 0.1 μmol/mL. 5: Mix 500 μL of the 0.1 μmol/mL solution with 500 μL distilled water to obtain 0.05 μmol/mL. 6: Mix 500 μL of the 0.05 μmol/mL solution with 500 μL distilled water to obtain 0.025 μmol/mL. 3) Reaction Setup Add the following reagents sequentially to centrifuge tubes under light-protected conditions: Test tube: 50 μL sample, 200 μL Reagent 1, 50 μL Reagent 2, 100 μL Reagent 3, and 150 μL Reagent 4. Control tube: 50 μL sample, 50 μL distilled water, 200 μL Reagent 1, 100 μL Reagent 3, and 150 μL Reagent 4. Do not add Reagent 2. Standard tube: 50 μL standard dilution, 200 μL Reagent 1, 50 μL Reagent 2, 100 μL Reagent 3, and 150 μL Reagent 4. Blank tube: 50 μL distilled water, 200 μL Reagent 1, 50 μL Reagent 2, 100 μL Reagent 3, and 150 μL Reagent 4. Mix thoroughly and react at 37°C protected from light for exactly 30 min. Then add 450 μL distilled water to each tube. 4) Absorbance Measurement Transfer the reaction solution to a 1 mL glass cuvette and measure absorbance at 450 nm. Record the values as A_test, A_control, A_standard, and A_blank. Calculate ΔA_test = A_test - A_control and ΔA_standard = A_standard - A_blank. A control tube must be prepared for every sample. The standard tube at each concentration and the blank tube only need to be measured 1-2 times. 5) Establishment of the Standard Curve Use 0.8, 0.4, 0.2, 0.1, 0.05, and 0.025 μmol/mL as the x-axis values and the corresponding ΔA_standard values as the y-axis values. Plot the standard curve and obtain the standard equation y = kx + b. Substitute ΔA_test into the equation to obtain x in μmol/mL. 3. Calculation of L-Lactate Content 1) Based on Tissue Protein Content L-lactate content (μmol/mg prot) = [x × (V_supernatant + V_B) × V_A] / (Cpr × W ×

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V_supernatant) = (1.1875 × x) / (Cpr × W). 2) Based on Tissue Sample Mass L-lactate content (μmol/g) = [x × (V_supernatant + V_B) × V_A] / (W × V_supernatant) = (1.1875 × x) / W. 3) Based on Bacterial or Cell Number L-lactate content (μmol/10^4 cells) = [x × (V_supernatant + V_B) × V_A] / (bacterial or cell number × V_supernatant) = (1.1875 × x) / bacterial or cell number. 4) Based on Liquid Sample Volume L-lactate content (μmol/mL) = [x × (V_supernatant + V_B) × (V_liquid + V_A)] / (V_liquid × V_supernatant) = 13.0625 × x. Variable Definitions: V_A: volume of Extraction Solution A added during extraction, 1 mL. V_supernatant: volume of supernatant transferred during extraction, 0.8 mL. V_B: volume of Extraction Solution B added during extraction, 0.15 mL. V_liquid: volume of liquid sample added during extraction, 0.1 mL. Cpr: sample protein content, mg/g. W: sample mass, g. Bacterial or cell number: expressed in units of 10^4. For 5 million bacteria or cells, enter 500. 4. Precautions 1) If A_test or ΔA_test is outside the linear absorbance range of the standards, proceed as follows: when the value is higher than the maximum, dilute the sample appropriately with distilled water and repeat the assay; when the value is lower than the minimum, prepare a more concentrated sample and repeat the assay. Modify the calculation accordingly. 2) Reagent 3 has a short period of validity after preparation. To facilitate experimental scheduling, one additional bottle of Reagent 3 is provided as a spare. Each bottle is sufficient for testing at least 25 samples. 3) The extraction solutions contain protein-precipitating components, so the prepared assay sample cannot be used for protein content determination. When L-lactate content is calculated using protein concentration, use a separate portion of the sample and prepare it with PBS or physiological saline by the same procedure before determining protein concentration. 4) Complete absorbance measurement accurately at the specified time point to ensure the accuracy and repeatability of the results. When many samples are tested, they may be assayed in batches to ensure consistent reaction times among groups. 5) The reagents in the reaction system must not be premixed in proportional amounts. They must be added sequentially in the order specified for the reaction system. 6) To

	ensure accurate results and avoid reagent loss, carefully read the instructions before the assay, confirm that reagent storage and preparation are adequate, and ensure that the operating steps are clear. Perform a preliminary assay with 2-3 samples expected to show relatively large differences. Obtain technical assistance promptly if problems arise during the procedure.
Storage	Kit Components and Storage: Extraction Solution A: liquid, 60 mL × 1 bottle; store at 4°C. Extraction Solution B: liquid, 10 mL × 1 bottle; store at 4°C. Reagent 1: liquid, 25 mL × 1 bottle; store at 4°C. Reagent 2: liquid, 140 µL × 1 tube; store at 4°C protected from light. Reagent 3: powder, 3 bottles; store at -20°C protected from light. Reagent 4: liquid, 20 mL × 1 bottle; store at 4°C protected from light. Standard: powder, 1 tube; store at 4°C.
Reconstitution	Reagent Preparation: Reagent 2: Before use, prepare Reagent 2 with distilled water at a volume ratio of 1:45. Prepare only the amount required immediately before use and mix thoroughly. Reagent 3: Before use, add 8 mL distilled water to each bottle and dissolve thoroughly. After aliquoting, the prepared reagent may be stored at -20°C for 2 weeks. Avoid repeated freeze-thaw cycles. Standard: Before use, add 1 mL distilled water and dissolve thoroughly to obtain a 100 µmol/mL L-lactate standard solution.
Reaction conditions	React at 37°C protected from light for exactly 30 min. Measure absorbance at 450 nm in a 1 mL glass cuvette.

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